REMARKS

The Official Action dated March 12, 2002 has been carefully considered. In view of the amendments presented herewith and these remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset, it is noted that a shortened statutory response period of three (3) months was set in the March 12, 2002 Official Action. The initial due date for response, therefore, was June 12, 2002. A Petition for a Three (3) Month Extension of Time is presented with this response, which is being within the three (3) month extension period.

It is noted preliminarily that claims 30, 32-35, 37 and 50-60 have been examined, in accordance with applicants' response to the earlier restriction and election requirements, and that claims 31 and 49 are withdrawn from consideration in this application. Applicants once again reserve their right to file one or more divisional applications on the subject matter of the non-elected claims in accordance with 35 U.S.C. §121.

As another preliminary matter, it is noted that the drawings accompanying this application are objected to by the Examiner and new formal drawings are required. This drawing objection and requirement for new drawings is believed to be improper with respect to this application, which is a U.S. national phase application under the Patent Cooperation Treaty (PCT), and duly filed by applicants in accordance with 35 U.S.C. §371. Inasmuch as there was no drawing objection during international proceedings under the PCT, the United States Patent

and Trademark Office is without authority to object to applicants' drawings. Such an objection is precluded by the following language of Article 27 of the PCT:

> No national law shall require compliance with requirements relating to the form or contents of the international application different from or additional to those which are provided for in this Treaty and the Regulations.

It is respectfully requested, therefore, that the drawing objection and requirement for new formal drawings be withdrawn.

It is further noted that the application is objected to based on a purported failure to comply with the requirements of 37 C.F.R. §§1.821-1.825 "for the reason(s) set forth in the attached Notice to Comply With Requirements For Patent Application Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures" (hereinafter the "Notice to Comply").

The Notice to Comply that is attached to the March 12, 2002 Official Action points out that sequences appear in Figure 4 and at pages 35 and 36 which do not have SEQ ID Nos. The Notice to Comply also indicates that applicants must provide an initial or substitute computer readable form (CRF) of the "Sequence Listing", a paper copy of the "Sequence Listing" (with amendment directing its entry into the specification) and the statement required by 37 C.F.R. §1.821(e) or (f) or (g) or §1.825(b) or (d). As to the last-mentioned requirements, it is noted that on August 2, 2001, applicants submitted a paper copy of the sequence listing, together with a CRF, an amendment directing its entry into the specification and the required

statement regarding the identity of the paper copy of the sequence listing and the CRF (the "Statement of Identity"). According to information provided in the United States Patent and Trademark Office's Patent Application Information Retrieval (PAIR) database, applicants' August 2, 2001 sequence listing submission was received by the PTO and the CRF was determined to be "Good Technically" and was "Entered into Database". That being the case, it is not understood why applicants are again being required to submit a CRF, a paper copy of the sequence listing and amendment together with a Statement of Identity. The Examiner's clarification of these requirements would be sincerely appreciated.

As can be seen from the foregoing amendments, SEQ ID Nos. have been added to the specification where appropriate. In view of the insertion of SEQ ID Nos. in the Brief Description of the Drawings section of the specification, applicants have refrained from inserting SEQ ID Nos. in Figure 4. This is in keeping with the following provision of §2422.02 of the Manual of Patent Examining Procedure:

> [W]hen a sequence is presented in a drawing, regardless of the format or the manner of presentation of that sequence in the drawing, the sequence must still be included in the sequence listing and a sequence identifier (SEQ ID No: X) must be used, either in the drawing or in the Brief Description of the Drawings [Emphasis added].

Thus, the present application, as now amended, is believed to comply fully with the requirements of 37 C.F.R. §§1.821-1.825.

The March 12, 2002 Official Action also includes an objection to the form of claim 52. According to the Examiner, claims 51 and 52 are drawn to identical subject matter. This objection is respectfully traversed. Claim 51 calls for a nucleic acid molecule encoding the amino acid sequence shown in the relevant portion of Figure 4. It is clear that, because of the degeneracy of the genetic code, this claim covers a genus of sequences, albeit ones that can be clearly identified as such by those skilled in the art. Claim 52, on the other hand, recites the specific nucleotide sequence shown in Figure 4, which is a particular member of the group of sequences claimed in claim 51. Therefore, these two (2) claims are not drawn to the same subject matter, as they differ in scope, and claim 52 properly depends from claim 51.

Turning to the substantive aspect of the March 12, 2002 Official Action, claim 34 is rejected under 35 U.S.C. §112, first paragraph, based on alleged inadequate enablement, due to the lack of deposit of vector pJP7. According to the Examiner, applicants are required to amend the specification to conform to the requirements of M.P.E.P. §6080.01(p)(C), which requirements are set forth at page 5 of the Official Action.

Claims 30, 32-35, 37 and 50-60 are also rejected under 35 U.S.C. §112, first paragraph, based on alleged inadequate enablement. After considering the various "Wands factors", the Examiner concludes that undue experimentation would be required to practice the claimed invention. This objection appears to arise because no actual data are presented in the specification

to show that the Reg protein does in fact regulate expression from the ohp promoter, and the Examiner apparently does not consider it reliable solely to infer function from sequence data.

Claims 30, 32-35, 37 and 50-60 have also been rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. This rejection is clearly overstated, in that the only claims that are specifically found objectionable are claims 50 (based on the recitation "or a modification thereof"), 57 (based on improper dependancy) and 58 (based on the absence of positively recited method steps in claimed method of use of a vector).

In accordance with the present amendments, the specification has been amended to incorporate SEQ ID Nos. where appropriate, as previously noted.

Regarding the claim amendments, claim 34 has been amended to further characterize the vector of claim 33 as comprising luxABsignalgenes, sacBgene, kanamycin, thiostrepton resistance genes, *E. coli* origin of replication and RP4 mobilizing elements. Support for this amendment is provided in Figure 6, together with the description of pJP7, at page 40, line 31 through page 41, line 13 of the specification.

Also in accordance with this amendment, claim 50 has been amended by deleting the terminology "or a modification thereof", claim 57 has been amended so as to be dependent on claim 56, rather than claim 50, and claim 58 has been canceled in favor of new claim 61. New claim 61 is drawn to the subject

matter of original claim 58 and positively recites the step of "transforming said host cell with a vector as claimed in claim 56".

An "Abstract of the Disclosure" in conformity with the requirements of 37 C.F.R. §1.72(b) is also submitted herewith.

No new matter has been introduced by reason of any of the amendments presented herewith.

As a result of the foregoing claim amendments, any indefiniteness that may have been engendered by the original form of claims 50 and 57 has now been eliminated. Furthermore, any indefiniteness that may have been engendered by the original wording of claim 58 has been eliminated in new claim 61 by the addition of the positive recitation of a method step, as noted above. Thus, the only matters remaining to be addressed are the 35 U.S.C. §112, first paragraph rejections of claims 30, 32-35, 37 and 50-60 and the separate rejection of claim 34 based on alleged inadequate enablement. These last-mentioned grounds of rejection are respectfully traversed.

1. Vector pJP7

As previously noted, claim 34 has been amended to recite a vector having the characteristics of pJP7 described in the specification. It is respectfully submitted that it would be well within the capability of one skilled in the art to construct a vector having such characteristics. Accordingly, a deposit of this vector is not necessary to satisfy 35 U.S.C. §112, first paragraph, in this case. It is, therefore, respectfully requested that this rejection be withdrawn.

2. Activity of ohp Operon Regulatory Protein

Regarding the §112, first paragraph rejection of claims 30, 32-35, 37 and 50-60, the Examiner argues that "the only way to demonstrate that a putative protein regulates expression from a promoter is to actually produce the regulatory protein and test for it's [*sic*] regulatory activity with the desired target promoter sequence". It is asserted that this view is explicitly supported by EP 719 862 and EP 713 914.

The Examiner further contends in this connection that Li *et al.* (at page 6416 col. 2) teach "the inability to predict the presence of a regulatory protein from inferential data and from comparisons to similar operons from other bacteria".

Applicants vigorously dispute the Examiner's limited assessment of the relative skill of those in the art, the predictability or unpredictability of the art and the conclusions drawn from such assessments. None of the cited references suggests the universal lack of predictability attributed to them by the Examiner. It can only be reasonably concluded that in none of these cases was the regulator gene identified by inferential data. The fact that general inferential principles either were not used (or could not be used) in three specific cases does not mean that such principles cannot be reliably used in other cases.

In fact the prior art has demonstrated principles relating to the structure and organization of catabolic operons, and sequences of prokaryotic DNA-binding proteins/regulatory proteins, application of which would lead the skilled person to

expect the Reg protein to regulate expression from the ohp promoter, as the following discussion will make clear.

a. Operon Structure

Structurally, an operon is two or more open reading frames which are closely adjacent and are co-transcribed from the same promoter. Transcription is controlled through protein-DNA interactions at a DNA sequence located close to the promoter, called an operator. Once transcribed, translation of the first gene is coupled to translation of the second by dint of overlapping or closely spaced stop/start codons. At the genetic level, an operon exhibits polarity; mutants in the first gene also affect downstream wild type genes. The polarity phenomenon operates through the translational linkage. A key feature of operons is their genetic linkage. This means that the regulatory elements, promoter, operator and operon structural genes are usually adjacent to one another i.e. the regulatory gene lies at the start of or end of the operon gene sequences.

Operons are very well characterized bacterial genetic structures (Jacob *et al.*, 1960, Comptes Rendus des Seances de L'Academie des Sciences 250:1727-1729; Jacob and Monod 1961, J. Mol. Biol. 3:318-356). Each operon is responsive to a specific regulatory protein which binds the operator region (usually a diad symmetry sequence adjacent to the promoter) allowing coordinate expression of the enzyme pathway genes. Thus their expression can be tightly linked to the presence/absence of a particular metabolic signal, usually a specific carbon source. The paradigm system is the *Escherichia coli* K12 Lactose (*lac*)

operon, which encodes a single regulatory gene *lacI* and three structural genes *lacZ* (beta-galactosidase), *lacY* (galactose permease), and *lacA* (thiogalactoside transacetylase) separated by the *lac* promoter and *lac* operator region. The *lacI* gene is codirectional with the *lacZYA* operon.

Prediction of gene function from DNA sequence features is accepted as the primary means of annotating sequence information. The methodology followed in the present application was standard bioinformatic practice (Archer 1985 PhD Thesis University of Glasgow "The colicinogenic plasmid ColK"; Morlon *et al.*,1988 Mol. Gen. Gene 211 (2): 231-243; Boyd *et al.*, 1989 Mol. Gen. Genet. 217 (2-3): 488-498) at the time and has not been significantly changed today. With respect to the *Rhodococcus* spp V49 *ohp* region, assignation of predicted functions were made using criteria that were and remain accepted practice in sequence annotation. In brief, this was an initial data analysis pass to identify open reading frames with coding potential based on a variety of different sequence features, followed by a second analysis pass of combined prediction based on protein sequence conservation, location and catalytic function (Powell and Archer 1998, Antonie Van Leeuwenhoek 74:175-188). The predictions for each proposed gene were made on the basis of a combination of eight interlocking bioinformatic tests.

These predictions were as follows: *ohpR* ohp regulatory protein; *Pohp/ohpO* promoter operator region; *ohpA* transport gene; *ohpB* monooxygenase gene; *ohpC* HMSH gene; *ohpD* catechol 2,3-dioxygenase gene. These predictions were subsequently proven by

enzyme assay of individual open reading frames (Powell and Archer 1998). Restriction fragments encoding OhpB, OhpC and OhpD (monooxygenase, hydroxymuconicsemialdehyde hydrolase and catechol 2,3-dioxygenase, respectively) were subcloned under the transcriptional control of the *lac* promoter in the *E. coli* cloning/expression vectors pUC18/19 and assayed in cell free extracts (Powell and Archer 1998, Antonie Van Leeuwenhoek 74:175-188). The predicted functions and their actual functions were correct in each case and identified ortho-hydroxyphenylpropionic acid as the preferred catabolic substrate. Thus the basis for the bioinformatic predictions was validated.

b. Regulator Protein

The ohpR reading frame was recognized as potentially coding on the basis of its length, codon preference, ficket plot and homology to known regulatory proteins, most significantly the GntR family of bacterial regulatory proteins typified by the helix-turn-helix (HTH) motif of the gluconate operon repressor GntR.

The helix-turn-helix structural motif was first demonstrated functionally, genetically and structurally as a DNA binding domain in the early 1980s (Sauer *et al.*, 1982 Nature 298:447-451; Pabo *et al.*, 1982, Nature 298:443-447 and Nature: 298L 441-443). As DNA sequence data accumulated, the HTH family of regulators was further subdivided into different subfamilies of HTH proteins. Haydon and Guest first described the helix-turn-helix GntR family of bacterial regulators of which OhpR is a member in 1991. They presented them as transcription factors

sharing a similar N-terminal DNA-binding (D-b) domain, but they observed near-maximal divergence in the C-terminal effector-binding and oligomerization (E-b/O) domain.

Subsequent genomic studies (Perez-Rueda *et al.*, 2000, Nucleic Acids Res 28(8):1838-47; Perez-Rueda *et al.* 2001, Mol. Evol. 53, 172) have reinforced the original concept that the helix-turn-helix motif is *prima facie* evidence of DNA binding domain. Furthermore, it has been reported that "*about 95% of all transcription factors described so far in prokaryotes utilize the HTH motif to bind their target site at the DNA. The remaining 5% is contributed by other binding motifs such as zinc fingers, helix-loop-helix (HLH), beta-sheet antiparallel, and RNA binding motif*" (Pérez-Rueda and Collado-Vides 2001 Nucleic Acids Res 28(8):1838-47). It is clear, based on the accumulated knowledge in the art, that within the bacterial kingdom, an HTH domain is sufficient to assign a DNA binding role to the protein involved.

Thus, on the basis of standard bioinformatic analysis, particularly protein sequence conservation between OhpR and known DNA binding regulatory proteins in a wide range of different bacterial species, it is clear that *ohpR* encodes a regulatory protein. This, combined with the position of ohpR relative to ohpABCD, is consistent with its being the regulator for the *ohp* operon.

Furthermore, there is an absence of promoter-like sequences between open reading frames ohpABCD within the structural gene cluster, and a promoter-like sequence is present immediately 5' to the cluster with significant regions of dyad

symmetry within the putative operon promoter region, entirely consistent with an operator sequence (*Pohp/ohpO*).

Therefore by combining the bioinformatic evidence, one skilled in the art would predict with very high confidence, solely from the sequences provided in the present application, that the *ohpR* gene regulates *ohpABCD* via interactions at *Pohp/ohpO*.

In summary, then, the disclosure of the sequence and context of orfR would lead the skilled person logically to the conclusion that orfR would be likely to encode a regulatory protein for the ohp operon. This is exactly what the present specification teaches. The specification does not invite those skilled in the art to identify the regulator protein for themselves, but provides a coding sequence for the protein (orfR, from position 295 to 1035), and the sequence of the region containing the promoter (in the region between orfR and orfT, i.e. between nucleotides 1036 and 1449 - page 40, lines 14 to 16 and Figure 3), and contains specific information about their respective functions, which is confirmed by the disclosed data. Thus, the conclusion necessarily follows that the specification is enabling. Both the protein and the promoter are provided to those skilled in the art by provision of the relevant nucleotide sequences along with information about suitable sources for their cloning. The functions of these elements are explained, and there is credible evidence in the specification to satisfy those skilled in the art.

Of course, in the event that those skilled in the art wish to demonstrate this for themselves, ample guidance is provided in the specification regarding suitable experiments which could be performed to demonstrate that the protein and promoter cooperate as stated. This is acknowledged by the Examiner in considering the amount of direction or guidance presented.

The adequacy of enablement provided by the present specification is clearly confirmed by the Declaration of John Archer submitted herewith. Dr. Archer's declaration describes experiments which establish that the region ohpR/Pohp encodes an OHP sensitive regulatory element and promoter and that expression from Pohp is driven by the OhpR(REG) protein. It is further averred in Dr. Archer's declaration that the *PohpR/ohpR/ PohpABCD* fragment used in these experiements, which encodes the regulator and contains the promoter/operator region, corresponds to nucleotides 1 to 1864 of the sequence shown in Figure 4 of the present application. The experimental results set forth in Dr. Archer's declaration clearly demonstrate that the REG protein switches on expression from a promoter of the ohp operon, located between the regulator and transport genes, all as described in the present application.

In any event, the literature contains many disclosures of similar experiments, e.g. EP 719 862 and EP 713 914, as pointed out by the Examiner. It would therefore be well within the ability of one skilled in the art to test the regulatory properties of the REG protein in relation to the ohp promoter,

even without the additional guidance provided by the present application. *In re* Wands explicitly states that "[e]nablement is not precluded by the necessity for some experimentation such as routine screening". It is respectfully submitted that any experimentation that may be required here is purely routine in light of the state of the art, and especially so in light of the teaching of the specification. Dr. Archer's Declaration speaks directly to the Examiner's "undue experimentation" allegation, pointing out that methods to determine whether or not a protein regulates expression from a particular promoter are routine and commonplace in the art and that no specific guidance over and above that provided in the present application would be required by one of ordinary skill in the art in order to confirm the activity of the OhpR protein and the *ohp promoter*.

In summary, Dr. Archer's declaration establishes, on the basis of methodology routinely practiced in the art, that the REG protein does in fact regulate expression of a reporter gene under the transcriptional control of the ohp promoter. This declaration is clearly relevant to the question whether or not adequate enablement is provided in the present specification for practicing the claimed invention and must be given due consideration in resolving this question. Cf. Ex parte Mark, 12 U.S.P.Q. 2d. 1904, 1906-07 (U.S.P.T.O. B.P.A.I. 1989).

For all the foregoing reasons, the 35 U.S.C. §112, first paragraph rejection of claims 30, 32-35, 37 and 50-60 is untenable and should be withdrawn.

In view of the present amendments and the foregoing remarks, it is respectfully urged that the objections and rejections set forth in the March 12, 2002 Official Action be withdrawn and that this application be passed to issue, and such action is earnestly solicited.

Respectfully submitted,

DANN DORFMAN HERRELL and
SKILLMAN, P.C.
Attorneys for Applicant

By Patrick J. Hagan
Patrick J. Hagan
Registration No. 27,643

PJH:ksk
Enclosure: John Anthony Charles Archer